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DELAYED GERMINATION IN SEED OF ALISMA PLANTAGO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 194

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(WITH EIGHT FIGURES)

I. Introduction

The cause of delay in the germination of seeds of various water plants has been the subject of considerable discussion and some experimentation. MÜLLER (23) early found that seeds of *Eichhornia* and *Heteranthera* do not germinate without first being desiccated, and LUDWIG (26) demonstrated this for seeds of *Mayaca fluviatilis*. PFEFFER (23) says, "nor is it certain whether BROWN and MÜLLER are correct in assuming that previous drying is essential for the further development of *Chlamydomonas* or for the germination of the seeds mentioned." A. FISCHER (12) found that acids and bases arouse many seeds of water plants from their dormancy, and attributes the action of these reagents to an embryo rather than a coat effect. The following species were studied by FISCHER: *Alisma Plantago*, *Potamogeton lucens*, *P. pectinatus*, *Hippurus vulgaris*, *Polygonum amphibium*, *Scirpus lacustris*, *S. maritimus*, *Sagittaria platyphylla*, *S. sagittifolia*, and *Sparganium ramosum*.

Somewhat later, CROCKER (6) cast doubt upon FISCHER'S conclusion that the acids and bases are effective through their action on the embryos. It was found that the seeds of various water plants (*Alisma*, *Sagittaria*, *Eichhornia*, etc.) germinate readily in distilled water frequently changed, provided the seed coats are broken or removed with care not to injure the embryo. From this it would seem that the embryos are not dormant if the limiting effects of the coats are not present. EWART (**11**) confirms these results by finding that the seeds of *Sagittaria* germinate readily after abrasion with sandpaper. He also points out the fact that the effect of the acid or the base is not counteracted by the application of a sufficient amount of the other to neutralize it. This criticism is especially significant in the light of the finding reported later in this paper, that the coat is largely composed of pectic substances.

It is evident that the dormancy in the seeds of water plants is of a different type from that in the seeds of the haw (**9, 10**), apple, peach, etc. In these forms the naked embryos do not grow when they are supplied with all ordinary germinative conditions if they are not first after-ripened. OSTENFELD (**22**) finds, confirmatory of the much earlier work of GUPPY (**15**), that the passage through the alimentary canals of birds greatly improves the germination of various water seeds. He considers further work necessary to justify the conclusion of either FISCHER or CROCKER. This statement seems to be fully justifiable, for it is possible that the reagents used by FISCHER have their total effect through their joint action on coats and embryos, although the latter when coat-free are certainly not dormant. It is for the purpose of throwing more light on this question that the following experiments are reported. Up to date the work has been entirely limited to a study of the seeds of *Alisma Plantago*. Of forcing agents, hydrochloric acid mainly has been studied.

II. Material

The ripened fruit of *Alisma Plantago* as it is shed from the plant is an akene, from which the pericarp can be removed by slight rubbing of the fruit between the fingers. This structure seems to play no rôle in water-intake or delay in germination, hence, for convenience in detecting the quality of the seed and for avoiding errors due to the large inert mass, the fruits worked with were always freed from this structure, leaving only the seed proper. A single harvest of seeds thus prepared shows much variation between the individuals both in appearance and in physiological behavior. In color the seeds vary from a deep reddish brown to a light pink. As a general rule the intact dark seeds absorb water less rapidly and to much slighter total amount than the light-colored ones. With the coats broken or removed, they also show far less vigor in water absorption and in growth rate. For this reason, in the experiments here reported, light-colored seeds of plump appearance were selected chiefly. Even with this precaution to remove less vigorous ones, the seeds showed great variation in rate of water absorption and of growth.

The air-dry seeds freed from the pericarp retain a relatively large percentage of water. The amount approximates 16 per cent, varying with the humidity of the air and with the seed. The large percentage of water retained in contrast to many fatty seeds is probably related to the richness of the embryo in starch and of its walls in pectic substances, and to the high pectic content of the relatively thick coat.

When seeds were to be used with coats broken or partly removed, it was at first done by inserting a thin knife blade between the two arms of the embryo. This method did not prove desirable, for it left many coats intact, only splitting the two layers lying against the embryo from each other but not breaking them. It also often cracked the embryos at the arch. It was soon found that slight pressure of a knife blade at the micropyle end removed a quite definite cap of the coat from that end of the embryo. The break occurs at thin regions well shown in fig. 1. In some experiments, the coats were also removed by similar means from the end of the embryo adjacent to the micropyle. In this case the breaking was somewhat more irregular, and the size of the pieces showed more variation, although at this end of the embryo the coat shows very definite thinner places at the position of the cross-walls, as is the case at the micropyle end. The feature just described also accounts for the method by which the embryo breaks the coat after the seed is treated with an acid or a base. In this case the same sort of cap is pushed off the micropyle end of the coat by the lengthening embryo. Before embryos from which a portion of the coat was removed were used, they were examined with the microscope to see that no injury was incurred in the process.

All the cultures were made under water or other solutions mentioned. The seeds will not grow normally between filter papers or on wet absorbent cotton, probably owing to insufficient water supply.

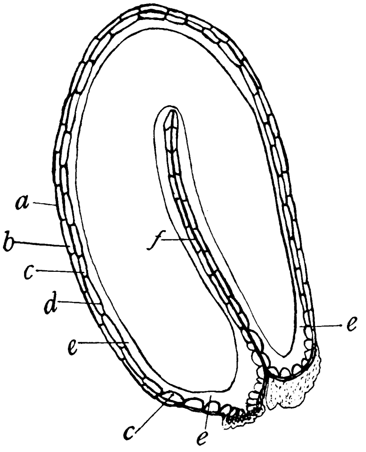

FIG. 1.—Camera drawing of a longitudinal section of the seed coat of *Alisma Plantago: a*, outer wall of the outer layer of cells, pectic substances swelling up and slowly dissolving in water; *b*, contents and cross-walls of the outer layer of cells, largely pectic material; *c*, contents and cross-walls of the inner layer of cells, largely pectic material; *d*, inner wall of inner layer of cells slightly suberized; *e*, acellular inner layer of coat, possibly originating from the endosperm, a mixture of pectic substance and hemicellulose.

III. Experiments

A number of experiments showed that the embryos themselves have no period of dormancy or need of after-ripening, for fruits just gathered from the plants gave prompt germination when the seed coats were ruptured and the seeds placed in water. Likewise, acid or base-treated seeds never showed as high percentage of germination (within 10–30 per cent) as seeds from which a portion of the coat was carefully removed. In short, there is no evidence of stimulation by the acid and base; they are simply more or less efficient agents for removing an obstruction to germination, never equaling in efficiency the mechanical method.

Intact seeds that have been soaked in water for 12 hours and then transferred to 2 mol. sodium chloride solution show rapid shrinkage, reaching a constant weight which they maintain as long as they are in the solution. These determinations are subject to rather large errors owing to the great surface represented by the 400 small seeds used in each test and the error of drying this surface to the same degree before each weighing. The permanent shrinkage in salt solution may be due to a semipermeable membrane in the seed coat or to a change in the water-imbibing power of the colloids of the coats and perhaps of the embryo owing to the presence of the salt. A. FISCHER found that the intact fruits of *Sagittaria* were not at all injured by five days' soaking in mol. copper sulphate solution. We find that one month's soaking of intact seeds of *Alisma* (1 mol. $CuSO_4$) does not injure their germination, if they are thoroughly washed and the coats opened before they are germinated. The opened seeds grow better in 0.000001 mol. $CuSO_4$ than in distilled water, but very little in 0.001 mol. $CuSO_4$. Soaking of the intact seeds in 10 per cent $AgNO_3$ for 24 hours kills all of them. From these facts it seems probable that in *Alisma* and *Sagittaria* the seed coats bear semipermeable membranes akin to those found in *Xanthium* (29) and various grasses (5, 28).

1. STRUCTURE AND MICHROCHEMISTRY OF THE SEED COAT.—As the later experiments will show, the study of the structure and the microchemistry of the coat is much to the point. Fig. 1 is a camera drawing of a median longitudinal section of the coat. In the main the coat consists of two layers of cells: the outer yellow, red, or brown layer (*b*), and the inner colorless layer (*c*). At the micropyle end there is usually only one layer of cells. In the region where the two arms of the seed lie together, the two adjoining outer layers form a collapsed mass (*f*), while the cells of the inner layer are distinct. The single layer of cells at the micropyle end have deep indentations from the inner face at the cross-walls. As a consequence, the inner face in this region has a very undulating appearance. When the seeds germinate after acid treatment or when one picks off the coat at the micropyle end, a rather definite cap is removed. The cap seems to result from the breaking at one of the more outer of these indentations. Inside the cellular portion of the coat is an acellular lining mass of material consisting of a mixture of hemicellulose and pectic material. This layer is rather thick at the micropyle end of the embryo, and as thick or thicker at the opposite adjacent end of the embryo. In this connection it should be stated that it is common to find seeds with the outer layer of cells largely abraded lying in water in the dormant

condition. This shows that it is largely, if not entirely, the inner layer of cells of the coat, with perhaps the acellular layer, that is responsible for the failure to grow.

The methods given by TUNMANN (31) in his late work were followed in the microchemical tests. The following reactions justify the conclusions given in the description of fig. 1. After treating sections of the coat 48 hours with cuprammonia and then staining with ruthenium red, *a*, *c*, *e*, and part of *b* became red. Treatment for 48 hours with cuprammonia followed by 2 per cent ammonia dissolved all the section except *d* and part of *b*. Sudan III stained only the inner wall of the inner layer of cells. Chlorzinc iodide stained *a* violet and *e* blue. Chromic acid in 10 minutes dissolves everything except *d*, but after 16 hours *d* still remains intact. One half-hour's heating in 5 per cent hydrochloric acid dissolves a considerable part of *e*, especially at the hilum end and the small adjacent end of the coat. One-half hour's heating in 2.5 per cent sulphuric acid followed by treatment with IKI stains *e* blue; IKI alone gives no reaction in any part. Treatment with copper sulphate for 48 hours, followed by 2 per cent ammonia, dissolved the greater part of *c;* 2 per cent ammonia alone brings about no visible changes. Sections in water for 10 days showed the contents and most of the cross-walls in *c* dissolved, and the plano-convex cells at the large end swollen to about twice their former size.

These tests indicate that there is no cellulose in the coat. The blue coloration of *e* with chlorzinc iodide is probably due to the presence of hemicellulose rather than cellulose, as indicated by its insolubility in cuprammonia, its ease of dissolution by 5 per cent hydrochloric acid, and its ready transformation to amyloid by 2.5 per cent sulphuric acid.

It is evident that the coat is largely composed of pectic substances, with a suberized wall at *d* and an admixture of hemicellulose in *e*. The dissolution or marked swelling of *a*, the contents and cross-walls of *c*, and the plano-convex cells at the large end of the embryo, indicate that these are made of the type of pectic substances included under the terms pectic, parapectic, and parapectosic acids. Slight chemical transformation of these substances may easily weaken the coat, so that the imbibing embryo can rupture it, for we shall see later that the evidence is strong that the delay is due to the swelling force of the embryo being unable to break the restraining coat. We must bear in mind also that without any chemical decomposition acids and bases may increase greatly the water-absorbing power of the hydrophilous pectic and hemicellulose substances of the seed coats, but undoubtedly chemical transformations as well as physical are brought about in the acid and base-sensitive substances of the seed coat.

Heating the seeds to 99–100° C. for 0.5 hr. in 2 per cent HCl, followed by 0.5 hr. in 2 per cent NaOH, the usual method of dissolving out the middle lamella of cell walls, dissolves away most of the coat at the large end of the embryo and causes the embryo to break the coat and elongate 200–400 per cent. All regions of the coat were modified; in some cases the walls were apparently largely dissolved away, leaving a thin lamella, and in others gelatinized and greatly swollen. Some color dissolved out of the wall in the acid, but immediately upon transfer to the base the latter took on a deep red color. Heating to 99–100° in 2 per cent NaOH for 0.5 hr. gave similar results, and 0.5 per cent NaOH was almost as effective. Even at room temperatures, similar but less marked changes appeared in 2 per cent NaOH, and at this temperature 0.5 per cent caused noticeable exosmose of coat pigments and decomposition of the coats within a half-hour.

It is found that seeds treated with 0.3 N HCl for 1 hr. at 40° C. give 30–50 per cent germination in distilled water; much poorer germination in 0.000001 mol. $CuSO_4$, and none at all in 0.001 mol. $CuSO_4$. As has been pointed out, the embryos with coats partly removed germinate better in 0.000001 mol. $CuSO_4$ than in distilled water. It seems that the copper is rather readily absorbed by the gel-like coat and the consistency of the latter changed. The absorption is evidenced by the coat turning a dark dull brown rather rapidly in the stronger solution and slowly in the weaker. The rather plastic gel of the coat becomes hard and brittle and is easily cracked under the pressure of a scalpel. The hardening takes place to a more marked degree in untreated seeds soaked in water. Apparently this hardening of the coat gel increases the resistance of the coat to the enlarging embryo, and thereby prevents germination, even in the treated seeds. The facts reported in this paragraph show the danger of applying reagents to intact seeds and drawing conclusions from the results as to the effects the reagents have on the embryos.

2. WATER RELATIONS OF THE EMBRYO AND COAT.—In considering the water relations of the embryo and coat we will first examine the rate of absorption of water by the seeds under three different conditions: (1) seeds with the coats removed from the micropyle end of the embryo; (2) intact seeds untreated; (3) intact seeds treated with solutions of 0.2 N HCl for various periods. Of the six experiments performed only two will be given, since they represent the trend of the results. Table I gives the data for the first of these, and fig. 2 presents the results in the form of curves.

TABLE I

Showing percentage of increase over air-dry weight of *Alisma* seeds treated as follows: I, 400 seeds in water, coats broken; II, 400 seeds in water, coats intact; III, 400 seeds, coats intact, in 0.2 N HCl for 2 hours then transferred to water. Temperature 20° C.

No. of hours soaked	Percentage of increase on basis of air-dry weight		
	I	II	III
1	40	31.6	34
2	65	40	48
3	65	40	48
4	68	40	52
18	73	50	52
23	180	50	52

Table II gives the data for the second experiment, and fig. 3 gives the results in the form of curves.

Only the general features of the curves in these experiments are of value, because of the necessary errors involved in the experiment. The seeds are very small, and the 400 used in each culture have a large specific surface. Any variation in degree of drying before weighing gives considerable error. Another variation that appeared in these experiments was the promptness with which the acid treatment became effective. This accounts for the curve for intake by the acid treatment running close to the untreated ones in the first experiment and falling between the other two curves in the second experiment. In the first experiment very few of the embryos had broken the coat previous to the 43d hour, while

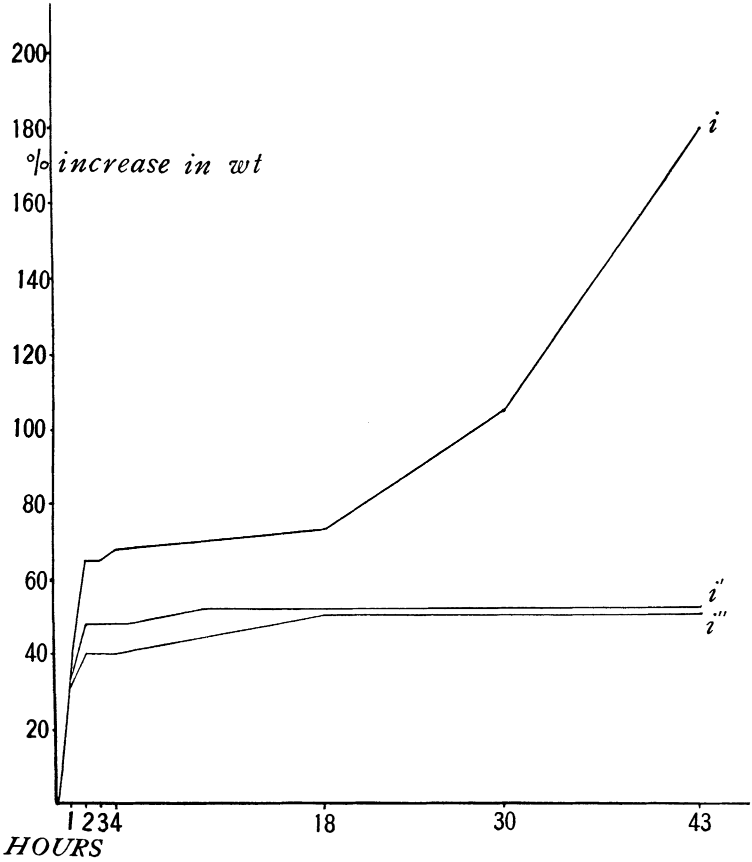

FIG. 2.—Rate of water absorption (percentage of increase over air-dry weight) by *Alisma* seeds with pericarp removed: temperature 20° C.; *i*, 400 seeds, coats broken and seeds soaked in water; *i'*, 400 seeds, coats intact, first two hours in 0.2 N HCl and rest of the time in water; *i''*, 400 seeds, coats intact and soaked in distilled water.

more than 30 per cent of them did so within 50 hours. In the second many of the embryos had broken the coats after 10 hours, and about half had broken the coats within 43 hours. The difference in the promptness of the action of the acid in these two experiments may be explained in part by the difference in temperature. When FISCHER used 0.2 or 0.3 N HCl with one or two hours' exposure as a forcing agent, he found 40° C. a very effective temperature. Individual seeds also show great variation in the readiness with which they swell to the maximum and begin growth. Old seeds, such as those used in the experiments with $CuSO_4$, seem more resistant to HCl. This is prominent even in cases where the coats are broken, as later results will show.

The curves for the absorption of water with the coats broken correspond closely in the two experiments reported, as they do in all the experiments. In these seeds the absorption of water is very rapid during the first 2–4 hours, amounting to 65–80 per cent of the air-dry weight, or to approximately 100 per cent of the dry weight. This is followed by a period of 10 or more hours of very slow water absorption, which in turn is followed by rapid water absorption. The first period of rapid water intake is largely a matter of imbibition and osmosis. The slow water absorption lying between these marks the interval between imbibitional and growth enlargement.

TABLE II

Showing percentage absorption of water (figured on the basis of air-dry weight) by *Alisma* seeds: I, coats broken, seeds in water; II, coats intact, seeds in water; III, coats intact, seeds 2 hours in 0.2 N HCl and then in water. Temperature 25° C.

No. of hours soaked	Percentage of water absorbed on basis of air-dry weight		
	I	II	III
1	32.5	23	45
2	55	33	52
6	80	45	53
9	80	45	61
21	101	51	....
32	160	51	73
52	....	....	96

With the untreated intact seeds the water absorption essentially all occurs within 2–4 hours, and approximates 45 per cent of air-dry weight, or about 70 per cent of dry weight. The imbibitional and osmotic capacity of the coat and embryo for water is not nearly satisfied, for, as is shown above, when the coat is broken water absorption is increased by about 50 per cent. From these facts, together with the structure of the seed, as well as from facts to be given later, one might be led to conclude that the embryo is hindered from swelling before its imbibitional forces are satisfied, because these forces are not sufficient to rupture the coat. The initial rate of water intake by both the intact seeds and the seeds with the coats broken equals that of the rapidly imbibing seeds of

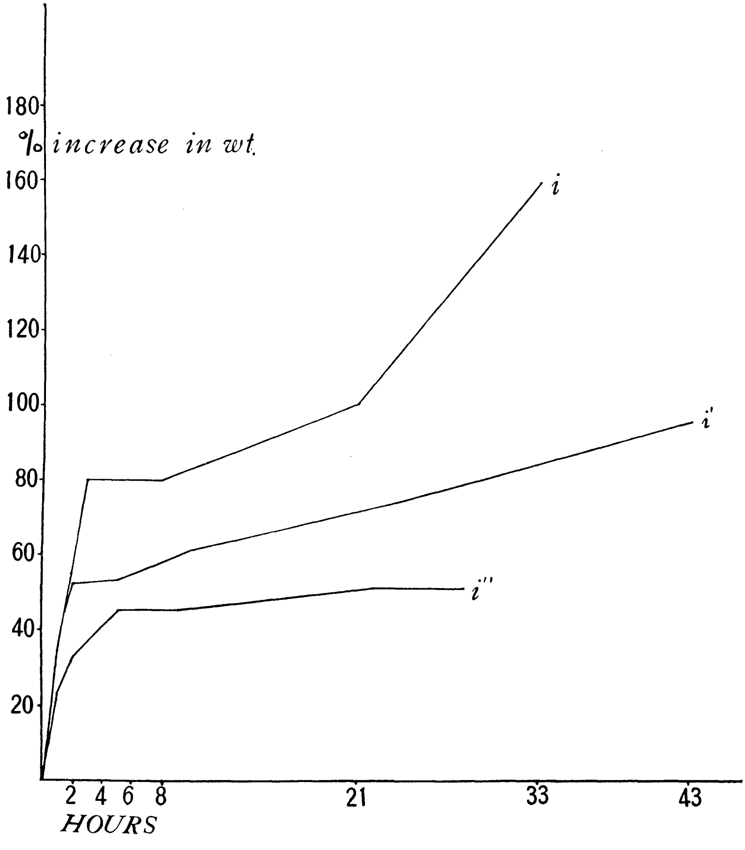

FIG. 3.—Rate of water absorption (percentage of increase over air-dry weight) by *Alisma* seeds with pericarp removed: temperature 25° C.; *i*, 400 seeds, coats broken and seeds soaked in water; *i'*, 400 seeds, coats intact, first two hours in 0.2 N HCl and rest of the time in water; *i''*, 400 seeds, coats intact and soaked in distilled water.

Xanthium as shown by SHULL (29), and far exceeds that of the slow imbibing seeds of the grasses as found by SCHRÖDER (28). In turn this rate of water intake is considerably exceeded by some of the Cucurbitaceae (32) (*Cucurbita argyrosperma*), where, however, the intake is largely due to a spongy capillary layer in the coat itself. On the other hand, we do not know to what degree the water intake in intact *Alisma* seeds is due to the coats; it is certainly due to this to a very large extent.

3. EFFECT OF ACIDS UPON RATE OF WATER INTAKE BY THE EMBRYOS.—MARTIN FISCHER (13) has found that bases and acids increase the rate as well as total amount of water absorption by colloids. BOROWIKOW (4) has shown that acids increase the rate of growth, or at least the rate of elongation of roots in water; while PROMSY (25) and ECKERSON (10) have shown that acids hasten the rate of germination of many seeds. These workers attribute the effect of the acids, at least in part, to the fact that they further the hydration of the colloids—in the case of the plant organs, the cell colloids. It will suggest itself immediately that the effect of the acids on the germination of water seeds may be due to their increasing of the imbibition force of the embryo, thereby enabling the embryo to rupture the coat. If this proves to be the case, the interesting situation appears that while the coat is the real cause of the delay, the effect of the acid is upon the embryo itself, increasing its imbibition power and enabling it to rupture the restraining coat. To determine this point experiments were made to find the rate of elongation of the embryo in water and in solutions of hydrochloric acid and sodium hydrate of various concentrations. In order to allow free access of the acid solutions and water to the embryo, the coat was removed at both ends of the embryo as described under "material." Only embryos that remained uninjured after the removal of the coats were used in the experiments. The first experiment was run with water, 0.01 N HCl, and 0.001 N HCl. Table III shows the rate of elongation of 20 embryos placed in water and measured at suitable intervals for 96 hours.

A great variation in the rate of elongation of the various embryos is shown. This seems to be characteristic of every crop gathered. Nos. 6, 13, and 20 are individuals showing low rate of elongation

TABLE III

Embryo	Length of embryo	Time in water and length of embryo (coats broken)						
	Dry	1 hr.	3 hrs.	6⅓ hrs.	23 hrs.	31 hrs.	50 hrs.	96 hrs.
1	95.5	104	112	117	140	140.5	145	360
2	100	115	121	125	138	141	152	396
3	95	105	117	126	146	146	148	230
4	97	103	117	121	121	122	124	161
5	87	96	114	128	142	142	148	200
6	96	101	114	128	141	138	146	147
7	99	117	127	132	151	151	157	800
8	99	109	118	129	168	199	230	430
9	89	107	111	124	137	134	134	360
10	85.5	89	99	107	113	113	116	165
11	90	104	118	116	116	115	120	230
12	94	101	117	129	143	143	170	560
13	86	106	116	119	143	144	146	144
14	93	95	118	124	134	140	157	570
15	84	114	122	121	121	123	124	430
16	82	89	101	103	114	136	139	180
17	74	88	97	106	118	116	120	270
18	100	111	125	141	185	210	276	540
19	83	190	116	124	132	132	136	400
20	95	111	122	124	132	134	136	140

TABLE IV

Embryo	Length of embryo	Time in 0.01 N HCl and length of embryo (coats broken)						
	Dry	1 hr.	3 hrs.	6⅓ hrs.	23 hrs.	31 hrs.	50 hrs.	96 hrs.
1	92	114	114	112	120	....	122	....
2	84.5	110	115	124	124	....	129	....
3	103	106	113	121	120	....	123	....
4	95.5	117	116	129	124	....	134	....
5	91.5	104	112	124	126	....	126	....
6	102	115	120	135	130	....	162	....
7	91	100	102	112	112	....	111	....
8	77.5	96	106	109	108	....	108	....
9	75.5	98	95	106	106	....	105	....
10	102	103	120	130	131	....	131	....
11	89.5	97	105	115	116	....	126	....
12	102	125	129	134	134	....	134	....
13	93	110	116	120	135	....	130	....
14	86	95	103	127	122	....	122	....
15	86	96	105	111	115	....	112	....
16	102	113	121	137	132	....	132	....
17	91	110	115	125	124	....	127	....
18	101	111	93	120	117	....	135	....
19	106	111	121	139	132	....	138	....
20	100.5	105	112	121	119	....	122	....

TABLE V

EMBRYO	LENGTH OF EMBRYO	TIME IN 0.001 N HCL AND LENGTH OF EMBRYO (COATS BROKEN)						
	Dry	1 hr.	3 hrs.	6⅓ hrs.	23 hrs.	31 hrs.	50 hrs.	96 hrs.
1........	83	99	100	108	103	....	106	106
2........	108.5	188	132	140	149	....	256	350
3........	85.5	103	108	112	127	....	129	145
4........	101	96	118	131	150	....	160	180
5........	92	109	120	125	120	....	122	125
6........	104.5	125	129	134	134	....	141	157
7........	98.5	107	115	122	130	....	130	144
8........	81	98	101	109	112	....	111	111
9........	87	101	109	120	125	....	125	125
10........	96.5	110	110	118	118	....	118	121
11........	98	125	127	132	131	....	132	137
12........	93	101	115	126	131	....	131	129
13........	90	112	125	129	126	....	128	125
14........	98	105	115	134	136	....	235	290
15........	87.5	98	114	110	114	....	119	110
16........	112	122	134	145	148	....	156	192
17........	85.5	95	111	119	122	....	122	156
18........	105	115	125	135	134	....	149	200
19........	96	101	114	123	120	....	121	127
20........	75.5	84	95	104	98	....	98	98

TABLE VI

Showing the elongation of 20 *Alisma* embryos each in water, 0.01 N HCl, and 0.001 N HCl. Coats removed at both ends of the embryo. Temperature 25° C.

Condition of the embryos	Total length of embryos	Average length of embryos	Percentage of increase over dry length
Air dry......................	1834.5	91.7	....
1 hour in water.............	2073.5	103.6	13.1
3 hours" "	2312.5	115.6	26.1
6⅓ " " "	2470.0	123.5	34.7
23 " " "	2759.5	137.9	50.4
31 " " "	2832.0	141.6	54.3
50 " " "	3025.0	151.2	64.9
96 " " "	6720.0	336.0	266.0
Air dry......................	1876.0	93.8	....
1 hour in 0.01 N HCl........	2104.0	105.2	12.2
1 hour in 0.001 N HCl.......	2257.5	112.9	20.3
6⅓ " " " "	2471.0	123.5	31.2+
23 " " " "	2462.0	123.1	31.2
50 " " " "	2512.0	125.6	33.9
	No further elongation		
Air dry......................	1878.0	93.9	....
1 hour in 0.001 N HCl.......	2131.0	106.5	13.4
3 hours" " "	2319.0	116.0	23.5
6⅓ " " " "	2474.0	123.7	31.7
23 " " " "	2515.0	125.7	33.9
51 " " " "	2789.5	139.5	48.5
96 " " " "	3139.0	156.9	67.1

and a low total at the end of 96 hours, while nos. 7, 22, and 14 show both a high rate and a high total. Table IV shows the elongation of 20 embryos in 0.01 N HCl; and table V the elongation of 20 embryos in 0.001 N HCl.

Table VI summarizes the results for the 20 seeds each in water, 0.01 N HCl, and 0.001 N HCl, and fig. 4 presents the results in the form of curves. The percentage of elongation is platted on the abscissa and the time on the ordinate. It is evident from these curves that the rate of elongation in water equals or exceeds that in either solution at all times, and shows a great advantage over both from 50 hours on. In the 0.001 N HCl cultures, the rate of elongation and the total elongation stands between that of water cultures and the 0.01 N HCl cultures.

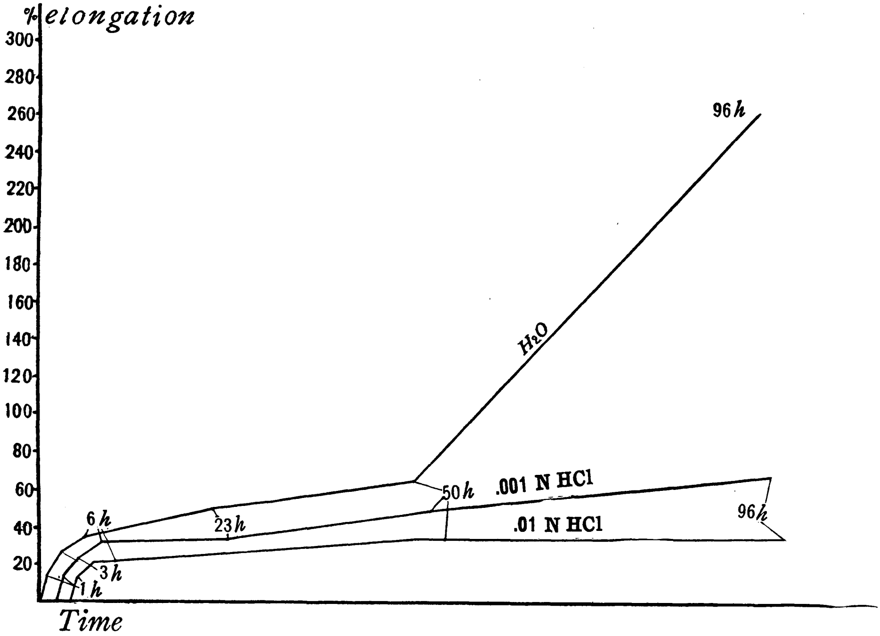

FIG. 4.—Rate of elongation of *Alisma Plantago* embryos in water, 0.01 N HCl, and 0.001 N HCl.

A second experiment was performed using water, N/3000, and N/6000 HCl. These results are summarized in table VII and put in the form of curves in fig. 5. In this case the N/3000 HCl at

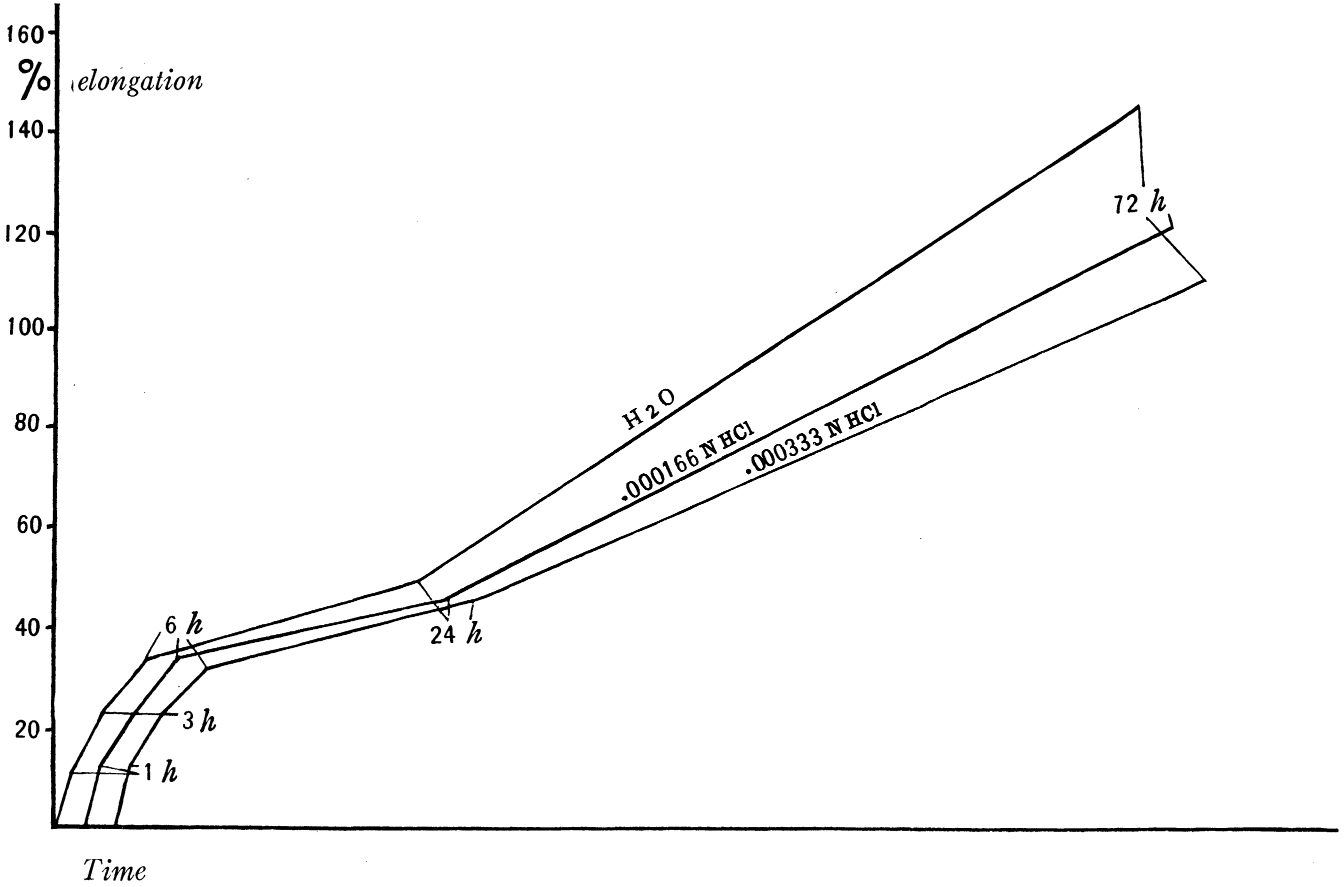

FIG. 5.—Rate of elongation of *Alisma Plantago* embryos in water, 1/3000 mol. HCl, and 1/6000 mol. HCl

first slightly leads water in rate of elongation, but falls behind it later. This slight early advantage can well be accounted for by the variation in the embryos from employment of so few, while the later falling behind water is undoubtedly related to injurious effects of the acid. The N/6000 HCl at first kept pace with water but later fell behind it.

TABLE VII

Showing the elongation of *Alisma Plantago* embryos in water, 1/3000 mol. HCl, and 1/6000 mol. HCl.

Condition of the embryos	Total length	Average length	Percentage of increase over air-dry length
Air dry......................	811	101.4	....
1 hour in water.............	899	112.5	10.5
3 hours" "	996	124.5	22.8
6 " " "	1080	135.0	33.2
24 " " "	1208	151.0	49.0
72 " " "	1967	246.0	142.5
Air dry......................	817	102.0	....
1 hour in N/3000 HCl.......	918	114.7	12.3
3 hours" " "	1035	117.0	26.6
6 " " " "	1102	137.7	35.0
24 " " " "	1193	148.9	45.3
72 " " " "	1788	223.5	119.0
Air dry......................	1005	100.5	....
1 hour in N/6000 HCl.......	1114	111.4	10.8
3 hours" " "	1222	122.2	21.5
6 " " " "	1312	131.2	30.5
24 " " " "	1449	144.9	44.1
72 " " " "	2107	210.7	109.0

Table VII A summarizes two other sets of measurements on the effect of N/3000 and N/6000 HCl on the rate of elongation.

TABLE VII A

Average percentage elongation for 10 embryos each in water and the HCl solution mentioned.

	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.
N/3000 HCl................	10.1	16.9	24.7	28.9	32.0
N/6000 HCl................	10.8	16.9	23.0	27.0	30.0
Water......................	9.1	16.2	21.9	27.5	30.6
N/3000 HCl................	11.0	20.0	26.0	29.0	32.0
Water......................	10.0	16.8	24.7	28.7	31.0

From all these measurements it seems barely possible that acid solutions hasten somewhat the rate of elongation of the embryo, and that the maximum effect appears at about N/3000 in a 5-hour period, but the effect is not great.

Table VII B shows the effect of various concentrations of NaOH on the rate of elongation of embryos. The solutions were protected from CO_2 of the air, but of course were continually being transformed to carbonates by the CO_2 from the respiring seedlings, so the concentration of the hydrate was falling from the beginning of the experiments.

TABLE VII B

Average percentage of elongation for 10 embryos each in water and the NaOH solutions mentioned.

	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.
0.01 N NaOH	12.9	17.8	23.5	28.2	30.5	43.0
0.001 N NaOH	12.7	19.2	24.9	32.6	35.7	49.0
Water	10.2	16.5	22.6	27.0	30.3	42.9
0.0001 N NaOH	11.9	18.9	25.0	30.7	34.7	46.9
N/3000 NaOH	11.9	18.0	24.2	27.6	30.4	40.3
Water	10.4	17.3	23.2	28.0	30.1	39.0

In each case the results in the various solutions should be compared only with their corresponding checks in water; also the checks in water are not comparable with each other, because the various experiments were not run at the same temperatures.

The base seems to increase imbibition rate very noticeably in contrast to acid, and there seems to be a distinct optimum concentration which for a 20-hour period lies in the region of 0.001 N NaOH. Such an optimum appears for the swelling of various colloid gels in the case of both acids and bases.

There is then doubtful evidence for acids causing increased rate of elongation of the embryo, and very certain evidence for bases doing so. It is probable, but by no means established, that along with such an increase in rate of elongation there goes an increase in force of elongation. If this be so, the base and perhaps the acid may further the breaking of the coat in part by an effect on the embryo.

4. TURGOR IMBIBITION AND GROWTH ELONGATION OF THE EMBRYOS.—The object of the experiments reported in this section

is to see to what extent the elongation of the embryos in water is a matter of imbibition and osmosis, and to what extent a matter of growth. Embryos with the seed coats removed at both ends were measured and placed in water for various lengths of time and the elongation determined. They were then transferred to plasmolytic agents and the amount of shrinkage determined. Cane sugar was finally chosen as the water-absorbing agent, for it produced permanent shrinkage and showed relatively low toxicity. Embryos which have been soaked in water for 12 hours and then transferred to 2 volume mol. cane sugar for 24 hours are still capable of normal growth when transferred to water. On the other hand, lithium chloride, which in saturated solution has great water-absorbing power, proved unsatisfactory both because of its toxicity and the variation in results.

Table VIII shows the average change in length caused in 8 embryos by soaking them 2.5 hours in water and then transferring them to 2 volume mol. sugar solution for 40 hours.

TABLE VIII

Elongation of 8 *Alisma* embryos soaked 2.5 hours in water, then transferred to 2 volume mol. cane sugar solution. Seed coats removed from both ends of the embryo. Temperature 25° C.

Treatment of embryos	Total length of embryos	Average length of embryos	Percentage of elongation
Air dry	814.0	101.8	....
2.5 hours in water	969.5	121.2	19.1
5 " " sugar solution	866.0	108.2	6.4
25 " " " "	856.0	107.0	5.2
40 " " " "	840.0	105.0	3.7
50 " " " "	840.0	105.0	3.7
Extreme individuals—		Length of embryo	
1. Dry	....	109	....
2.5 hours in water	....	139	27.5
40 " " sugar solution	....	111	1.9
2. Dry	....	96	....
2.5 hours in water	....	108	12.5
25 " " sugar solution	....	98	2.0
40 " " " "	....	98	2.0

Two and one-half hours in water gives an elongation of 19 per cent of the air-dry length, while the transfer to the sugar solution shrinks the embryos back to within 3.7 per cent of the air-dry length. The two extreme individuals are also given in the table. One showed elongation in water of 27.5 per cent and shrinkage in the sugar solution back to 1.9 per cent; the other extreme individual showed an elongation of 12.5 per cent in water and shrinkage back to 2 per cent in the sugar solution. In all the measurements of this section we find great variation between the individual embryos.

Table IX shows the elongation of 10 air-dry embryos, freed from the seed coats at both ends, when they are placed in 2 volume mol. cane sugar.

TABLE IX

Elongation of 10 *Alisma* embryos placed in 2 mol. cane sugar solution for 52 hours. Seed coats removed at both ends of the embryos.

Treatment of embryos	Total length of embryos	Average length of embryos	Percentage of elongation
Air dry	1080.0	108.0	....
5 hours in sugar solution	1093.0	109.3−	1.2+
20 " " " "	1109.7	111.0−	2.7+
40 " " " "	1113.5	111.3+	3.1
52 " " " "	1114.5	111.4+	3.2

After 52 hours these embryos showed an average elongation of 3.2 per cent. Allowing for considerable variation in individuals, it is evident that about the same final elongation results whether air-dry embryos are soaked directly in 2 mol. cane sugar or whether they are first soaked in water for 2.5 hours and then transferred to the sugar solution. The 19 per cent elongation of the embryo which occurs in water in the course of 2.5 hours is all a matter of imbibition and turgor. The intact seed itself, as we shall see in the next section, elongates on the average 10 per cent when it is soaked in water for a similar period. It is probable that the embryo in the intact seed thus soaked elongates far less than 10 per cent, for the acellular mass at both ends of the embryo and at the arch show much swelling in water, as do the other layers of the coats. Were the embryos in the intact seed allowed to carry on their imbibitional and osmotic intake of water freely and break off the coat cap at the large end of the embryo, within 2.5 hours the embryos would extend 10 per cent of their total length beyond the coat. Since the embryo has two arms of about equal length, the imbibitional and osmotic swelling would cause the embryo to extend 20 per cent the length of the seed beyond the limits of the coat. The rapid elongation of the freed embryos is brought about in part by the rapid swelling of the pectic walls. *These facts force one to believe that delay in germination in the Alisma seed is caused by the seed coat preventing the complete imbibitional and osmotic elongation of the embryo.* If one assumes that, on the average, the embryo in the soaked intact seed has elongated only 3–4 per cent of its air-dry length, and this is probably not far from correct, one sees that in the soaked condition of the seed the embryo is in water equilibrium with 2 volume mol. cane sugar solution. Such a solution has an osmotic pressure exceeding 100 atmospheres according to later estimates (**24, 27**). This probably gives a rough estimate of the force with which the embryo is pressing against the coat in the intact seed lying in water.

TABLE X

Elongation of 8 *Alisma* embryos soaked in water 5 hours and then transferred to 2 volume mol. cane sugar solution. Seed coats removed from both ends of the embryo. Temperature 25° C.

Treatment of embryos	Total length of embryos	Average length of embryos	Percentage of elongation
Air dry	803	100.4	
5 hours in water	1050	131.2	30.8
5 hours in sugar solution	879	109.9	9.5
25 " " " "	861	107.6	7.2
40 " " " "	863	107.9	7.5
Extreme individuals—		Length of embryo	
1. Air dry		97.0	
5 hours in water		131.2	40.2
5 " " sugar solution		116.0	19.5
25 " " " "		114.0	17.5
2. Air dry		103.0	
5 hours in water		128.0	24.2
5 " " sugar solution		110.0	6.8
25 " " " "		111.0	7.7

Table X shows the changes in length brought about in 8 embryos by soaking them 5 hours in water and then transferring them to 2 volume mol. cane sugar for 40 hours. Five hours in water gives an average elongation of 30.8 per cent, and in the extreme individuals

40.2 per cent and 24.2 per cent. Transfer to the sugar solution caused an average shrinkage back to 7.2 per cent elongation.

In the extreme individuals the shrinkage was back to 17.5 per cent and 7.7 per cent elongation. It is evident that permanent elongation or growth has set in to a slight but measurable degree at the end of 5 hours. In one of the extreme individuals the growth was considerable. This is another example of the great individual variation that is always evident. Fig. 6 shows these results in the form of curves.

Table XI shows the changes in length brought about in 14 *Alisma* embryos by soaking them 16 hours in water and then transferring to 2 volume mol. cane sugar for 25 hours.

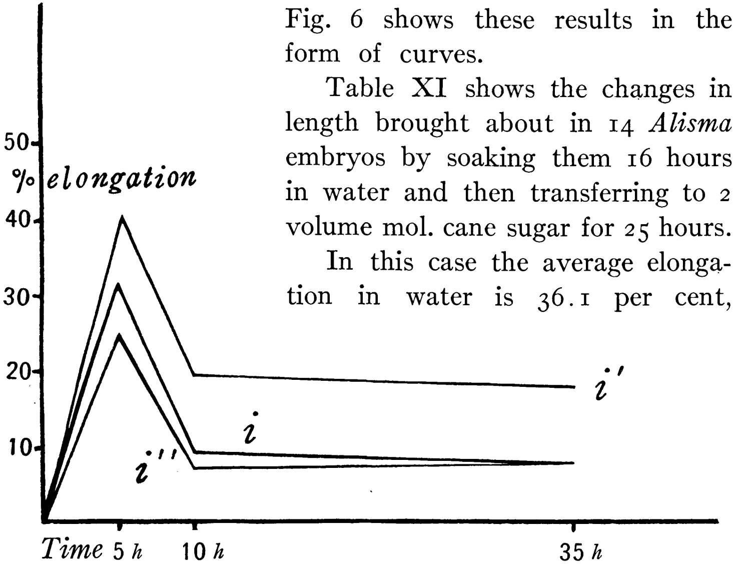

FIG. 6.—Percentage of elongation of *Alisma* embryos (seed coat removed at both ends) soaked in water 5 hours, then placed in 2 mol. cane sugar solution: measurements were made on dry embryos, after 5 hours in water, and after 5 and 30 hours in the sugar solution; *i*, curve for the 8 embryos studied; *i'*, curve for the individual showing the greatest swelling; *i''*, curve for the individual showing least swelling.

In this case the average elongation in water is 36.1 per cent, while the sugar causes a shrinkage back to 11 or 12 per cent elongation. The behavior of the extreme individuals is also shown in this table. Greater permanent elongation is evident than in the embryos in water for 5 hours. Here also there is some evidence of a slight permeability to sugar as shown by a slight swelling from 5 hours to 10 hours. Fig. 7 shows these data in the form of curves.

TABLE XI

Elongation of 14 *Alisma* embryos soaked in water 16 hours and then transferred to 2 volume mol. cane sugar solution. Seed coats removed from both ends of the embryos. Temperature 25° C.

Treatment of embryos	Total length of embryos	Average length of embryos	Percentage of elongation
Air dry	1378	98.4	....
16 hours in water	1876	134.0	36.1
5 " " sugar solution	1536	109.6	11.3
25 " " " "	1543	110.2	11.9
Extreme individuals—		Length of embryo	
1. Air dry	....	98.5	....
16 hours in water	....	149.0	51.0
5 " " sugar solution	....	115.0	16.7
25 " " " "	....	115.0	16.7
2. Air dry	....	102.0	....
16 hours in water	....	129.0	26.4
5 " " sugar solution	....	105.0	3.0
25 " " " "	....	108.0	5.0

Table XII shows the behavior of 13 *Alisma* embryos kept in water 16 hours and then transferred to 1 volume mol. cane sugar.

TABLE XII

Elongation of 13 *Alisma* embryos placed in water 16 hours and then transferred to 1 volume mol. cane sugar for 25 hours. Seed coats removed from both ends of the embryo. Temperature 25° C.

Treatment of embryos	Total length of embryos	Average length of embryos	Percentage of elongation
Air dry	1283.0	98.7	....
16 hours in water	1716.0	132.0	33.7
5 " " sugar solution	1535.0	118.0	19.0
25 " " " "	1565.5	120.4	22.0
Extreme individuals—		Length of embryo	
1. Air dry	....	87.0	....
16 hours in water	....	138.0	58.5
2 " " sugar solution	....	109.0	25.3
2. Air dry	....	94.0	....
16 hours in water	....	115.0	22.3
25 " " sugar solution	....	109.0	16.0

The 1 mol. cane sugar shows far less shrinkage than the 2 mol. Fig. 8 shows these data in the form of curves.

This series of experiments shows that the imbibition and osmotic forces alone cause an average elongation of about 20 per cent in the embryos partly freed from the coat. The elongation all occurs within 2.5 hours when the temperature is maintained at 25° C. This elongation of the embryo is probably three to five times that taking place when the intact seed is soaked for the same time or even for months. It is probable that the swelling of the embryo in thickness is inhibited to quite as large a percentage by the coat, for when one observes the embryo protruding from the coat, owing to the removal of the cap at either end, he sees a marked constriction at the line of the broken edge of the coat. The much swollen acellular layer seems to restrict greatly the swelling of the portion of the embryo within it. Growth begins within five hours on the average when partly naked embryos are placed in water at 25° C. Even 16 hours in water at this temperature shows rather slight growth, although the average elongation is about 36 per cent. This all emphasizes the main rôle of osmosis and imbibition in the early very rapid elongation of

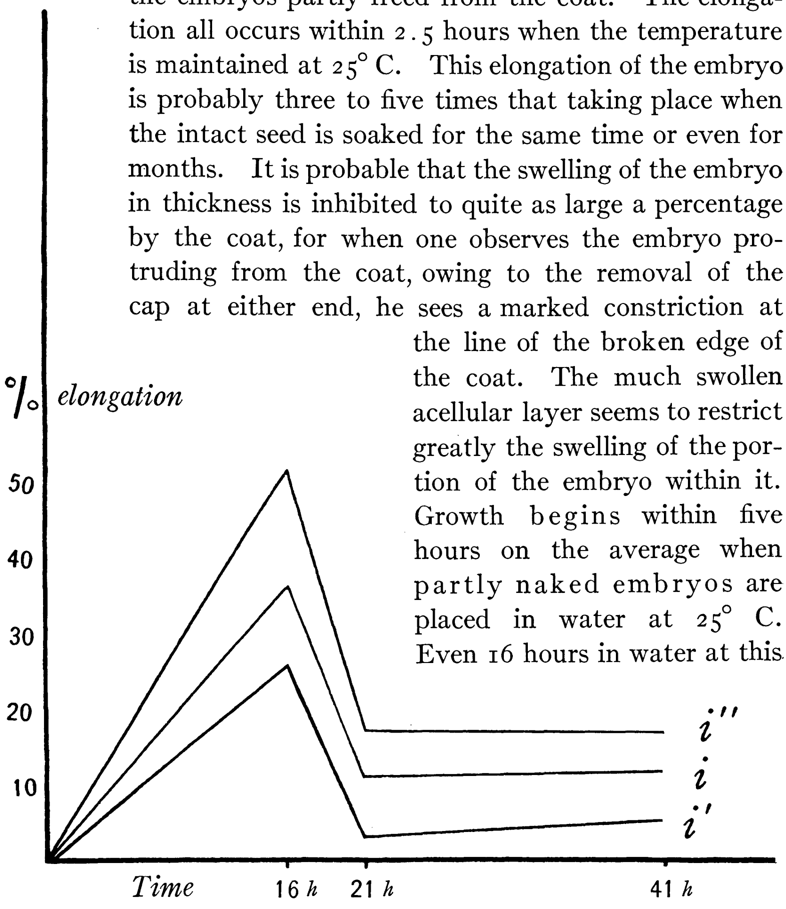

FIG. 7.—Percentage of elongation of 14 *Alisma* embryos with seed coats removed at both ends, soaked 16 hours in water, then placed in 2 mol. cane sugar solution for 25 hours: *i*, curve for the 14 embryos studied; *i′*, curve for the embryo showing the greatest swelling; i″, curve for the embryo showing least swelling.

the embryo. The great elasticity of the embryo as compared with that of the intact seed is also noteworthy. The results of this section enable one to approximate the force (imbibitional osmotic) with which the embryo of *Alisma* in the saturated condition is pressing against the coat; this approximates 100 atmospheres (24, 27).

ATKINS (34) finds in certain legume seeds that only imbibitional forces are at work taking up water until growth actually begins; that is, a plasmolytic agent (KNO_3) does not inhibit the rate or final total amount of water taken up. In *Alisma* seeds, whether the coats are intact or not, as is seen from the experiments in this and previous sections, semipermeable membranes are functional from the beginning. With the coats broken, it is very likely protoplasmic membranes that are functional. It is evident that ATKINS' findings are not of universal application.

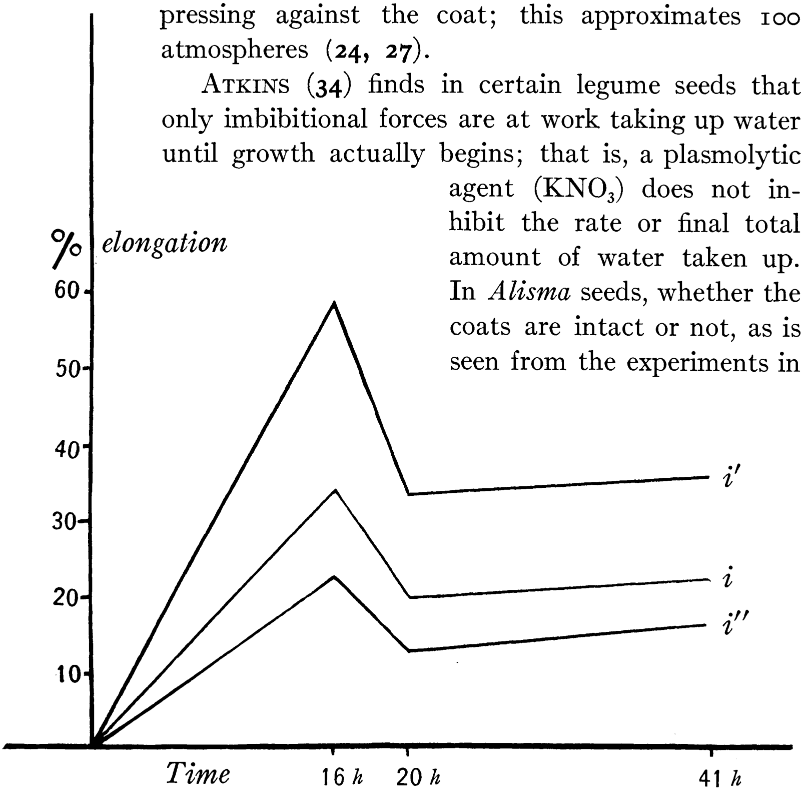

FIG. 8.—Percentage of elongation of 13 *Alisma* embryos (coats removed at both ends) placed in water for 16 hours, then transferred to mol. cane sugar solution for 25 hours: measurements made on dry embryos, after 16 hours in water, and after 5 and 25 hours in the sugar solution; *i*, curve for the 13 embryos studied; *i'*, curve for the individual showing most swelling; *i''*, curve for the individual showing least swelling.

5. ELASTICITY OF THE SEED COAT.—An attempt was made to determine the elasticity of the seed coat of *Alisma*. Thirteen good seeds freed from the ovary wall were selected and the length measured from the crown of the arch to the tip of the big arm and the width across the middle of the two arms. The seeds were soaked one day in water and remeasured and placed in 2 mol. cane sugar solution and measured after 15 and 24 hours. They were then transferred to water for one day and measured; then followed four days in dry air with the final measurement. Table XIII shows the modifications brought about in the two dimensions by the various treatments. The slight shrinkage caused by the transfer from water to cane sugar is especially noticeable. It shows the very slight elasticity of the seed coat as compared with the embryo. The total elongation of the intact seed, which is reached after two or three hours and becomes no greater even after months, is also slight (10 per cent) as compared with the embryo (19 per cent in 2.5 hours, 30.8 per cent in 5 hours, and 36.1 per cent in 16 hours).

TABLE XIII

Thirteen *Alisma* seeds, ovary wall removed, seed coats intact, soaked in water, followed by 2 volume mol. sugar solution, followed by water, and finally dried in air to get an estimate of the elasticity of the coats. Temperature 25° C.

TREATMENT OF THE SEEDS	WIDTH IN SCALE LENGTHS		LENGTH IN SCALE LENGTHS		PERCENTAGE OF INCREASE OVER DRY SIZE	
	Total	Average	Total	Average	In width	In length
Air dry	334.0	25.7	905.8	69.7	....	....
1 day in water	494.0	38.0	993.5	76.4	14.0	9.8
15 hours in sugar solution	481.0	37.0	963.0	74.1	11.0	6.3
24 " " " "	478.0	36.8	952.0	73.2	10.0	5.3
1 day " water	508.0	39.6	996.5	76.6	17.0	10.0
4 days " air	447.5	34.4	931.0	71.6	3.0	3.0

6. OXYGEN PRESSURE IN RELATION TO THE GROWTH OF ALISMA SEEDS AND SEEDLINGS.—We have already shown that the failure of *Alisma* embryos to break the coats, as well as their dormancy, is due to the fact that their possible imbibitional and osmotic swelling can be only in small part consummated in the intact seed. It has long been established (NOBBE, *Samenkunde*. 1876) that the swelling of seeds is merely a mechanical process which goes on

independently of oxygen supply. It is evident, therefore, that lack of oxygen cannot be the cause of dormancy in these seeds. MAZÉ (**20**) finds that seeds of most land plants will not germinate under water, and that storage under water in otherwise good germinative condition soon leads to loss of vitality. Good aeration of the water or addition of hydrogen peroxide to it will lead to germination under water. In contrast to this, GUPPY (**15**), FISCHER (**12**), and others have shown that seeds of many water plants may lie in water for years without germinating and still retain their vitality. This in itself suggests that the seeds of water plants are very different from land plants in their behavior toward a deficiency in oxygen. In *Alisma* we have shown that the embryo in the intact imbibed seed lacks much of being fully saturated with water. This probably holds for the seeds of many water plants, as one of us (**6**) has pointed out, and may in part account for the lack of injury from long storage under water. So far as investigated, seeds of water plants have extremely low minimum oxygen pressures for germination, if indeed any free oxygen is necessary for the process. The fact that their physiological processes can go on normally under water probably accounts in part for the capacity for water storage. One of the authors (**6**) has already pointed out this fact for seeds of several water plants.

During this investigation we have made a large number of experiments determining the relation of the amount of oxygen pressure to the growth rate, course of development, and chlorophyll formation in *Alisma* seeds and seedlings. We need mention only a few of the data and general results.

In all the experiments with the effect of various oxygen pressures on germination and development, the embryos used were partially freed from the coats and in an uninjured condition. Part of the experiments were run *in vacuo* and part by the displacement of air by hydrogen. In general, the cultures in hydrogen did not do as well as those *in vacuo*. This may be due to a slight toxicity of hydrogen itself or to some impurity in it. The hydrogen was derived from the electrolytic dissociation of water and freed from oxygen by passing it through a quartz tube filled with small copper disks and heated white hot with a blast flame. The hydrogen proved to be essentially free from oxygen, as shown by its failure to color potassium pyrogallate. The experiments *in vacuo* were performed as follows. The seeds freed from coat cap and soaked in water at 0° C. for 12 hours were placed in 30 cc. distilling flask bearing about 20 cc. of distilled water. The main neck of the distilling flask was sealed off just above the side neck, and the side neck reduced to a capillary tube at one region. A Geryk air pump (capable of exhaustion to less than 0.1 mm.), protected by a drying system with great water-absorbing capacity, was attached to the side neck and the flask exhausted. The flask was then placed under water at 35° C. for one-half hour and the exhaustion continued. During the process the water generally boiled down to 5 or 10 cc., depending upon the bore of the capillary region. While still maintaining the boiling and exhaustion, the capillary was carefully sealed off.

Since toxicity entered into the results with hydrogen, we will report only experiments *in vacuo*, and of these only 2 of the 30 or more cultures need to be given. In these experiments 10 seeds were used in each culture. The controls were in Stender dishes bearing 0.5 cm. column of distilled water. The cultures were run in total darkness at a temperature of 21° C. for 21 days.

TABLE XIV

Length of the seedlings *in vacuo*		Length of the seedlings in full air pressure	
I	II	I	II
40 mm.	35 mm.	70 mm.	70 mm.
30	35	60	60
30	35	60	60
30	40	50	60
40	40	50	60
25	30	45	55
25	30	45	55
25	30	40	50
32	30	40	50
25	15	40	50
Av. length. . 30 mm.	32 mm.	50 mm.	57 mm.

The imbibed embryo in the intact seed is approximately 2.5 mm. long, so that the total elongation in the vacuum cultures equals

28–30 mm., 1120–1200 per cent; while in full air pressure, other conditions the same, an elongation of 48–55 mm., or 1820–2200 per cent, occurs. In these conditions only the stored food of the embryo is available for growth. It is evident that there is a much more economical use of the food in the presence of oxygen than in its absence, and still the growth is very considerable in its absence. It is possible that the use of nutrient solutions would have induced much greater growth, for it is certain that much leaching (**35**) of the salts of the embryos must have occurred in these experiments.

Even in light, no leaf branches are ever formed in the seedling grown *in vacuo*, nor does any chlorophyll develop. In weak diffuse light at 25° C., the seedling did not develop chlorophyll even after a month, if the air pressure was less than 5 mm. If the air pressure exceeded 5 mm., chlorophyll generally developed in these conditions. This shows that the oxygen pressure demanded for greening is rather slight though easily measurable. Seedlings in darkness under full air pressure all develop one or more leaves. If the air pressure is reduced much below 5 cm., no leaf branches develop even after two months. If the air pressure is more than 15 cm., there is an abundant formation of leaf branches. The development of a primary root also seems to be dependent upon oxygen pressure. Nearly every seedling growing in a drop culture in a Van Tieghem cell shows the early development of a primary root, as do seedlings growing on the water surface. Fewer seedlings developing at the bottom of a considerable layer of water show primary roots, and none in cultures with air pressure reduced much below 5 cm. The growth of water seeds and seedlings *in vacuo* stands in contrast to the facts found by SHULL (**30**), BECKER (**2**), and ATWOOD (**1**) for the seeds of land plants, where definite oxygen minima exist.

IV. General discussion

Work to date has shown that delayed germination of seeds is secured in a variety of ways: by almost absolute exclusion of water by seed coats, as in the "hard-seeded" legumes and species of several other families; by limiting the degree of swelling of the embryo by surrounding structures, as in *Alisma* and probably in seeds of a large number of water plants; by reduction of oxygen supply below the minimum necessary for germination by means of seed or fruit coats or other structures, as is the case in *Xanthium* (30) and a number of other Compositae (2), in *Chloris ciliata* (14) and other grasses, and in *Datura Stramonium;* and finally, perhaps, by deficiency in salts. To these must be added delays due to embryo characters, as slowness of acid development in the hypocotyl of the haw, apple, peach, and perhaps other Rosaceae. The investigation of the mechanics of delayed germination in any particular seed should consider all of these possibilities, and not assume, under the incubus of the overworked stimulus conception with its indefiniteness and implied inscrutability, that the cause must lie in the embryo. The German investigators, with few exceptions, have been especially prone to the stimulus view and its implication that in characters of the embryo alone dormancy is determined. They of course except the seeds that are known as "hard-coated." This inclination to the stimulus conception is evident in FISCHER'S (12) interpretation of his results with seeds of water plants. We find BEHRENS (3) assuming that the pricking of intact seeds has its effect only through stimulus action, and not implying at all the possibility of greater oxygen admission or mechanical weakening of the coats. The same can be said of KIESSLING (17) working with various grasses, and KINZEL (18) with the "light-hardened" seeds of *Nigella*.

Again, in their work with light-sensitive seeds, with the single exception of GASSNER (14) on *Chloris ciliata*, the German investigators have assumed that the light has its effect always upon the embryo and never upon the coat. One must remember that the coat is most strongly exposed, and that it reduces the light intensity greatly before it reaches the embryo, at least in heavy coated seeds like *Phacelia tanacetifolia*. It is quite as possible that photochemical changes are occurring in the coat as in the embryo. NEUBERG has found that about every group of organic substances, polysaccharides, fats, proteins, etc., is hydrolized, and often the completely hydrolized products are further transformed by light, if iron, manganese, or certain other salts are present. These decompositions occur in non-living substances, so there is no reason for assuming that photochemical transformations are limited to the embryo and are not occurring in the more highly illuminated surrounding structures. Again, it is well established that light increases the permeability of protoplasmic membranes to various solutes. The possibility appears that it may likewise increase the permeability of non-living semipermeable membranes of the coat to oxygen or perhaps other materials needed for germination. Likewise, they have failed to see that the fixation of the carbon dioxide released in respiration may in some cases supply the oxygen needed for germination. It is strange that these workers have never done the evident thing of experimenting with naked embryos to see what their needs are in the way of germinative conditions. In seeds like *Viscum album* it is evident that the demand for light cannot be related to the seed coats, but in most light-sensitive seeds the conclusion is not evident.

It is established, however, that not all cases of delayed germination are due to coat characters acting in conjunction with embryo characters, but there are some embryos, or rather organs of some embryos, that are dormant when entirely naked. But even here it is probable that the explanation of the action of forcing agents will not need to be veiled under such an indefinite term as stimulus. ECKERSON (10) has shown that the hypocotyls of haw and apple seeds are dormant because of slowness in developing acidity, a condition necessary for proper water absorption for growth and for the formation and action of digestive and perhaps respiratory enzymes. The acid development begins even during swelling in all organs of most seeds, as it does in the cotyledons of the haw and apple, but the hypocotyls in the latter seeds remain slightly basic or neutral for a long period. It has been shown (9, 10) that temperature and water and oxygen supply determine to a great degree the speed of acid development in the hypocotyls mentioned. Likewise, the seed coats and carpel walls play an important rôle here, so far as they affect these factors. Acids are good forcing agents for dormant hypocotyls; apparently, they have their significance in this fundamental relation to water absorption and to the formation and activity of enzymes. LEHMANN (19) has suggested that light and agents and conditions that substitute for light in light-demanding seeds have their effect in bringing about hydrolysis of proteins. His evidence is hardly conclusive. It is quite possible, however, that dormancy in an embryo may be due to the abeyance of some single simple process under a given set of conditions. If so, it may be any one of several aside from protein hydrolysis. If such cases exist, they stand more or less in contrast with the case worked out by ECKERSON (10).

FISCHER found phosphoric and certain organic acids more effective as forcing agents for *Alisma* than their dissociation would insure on the basis of hydrogen ions. This we are unable to explain. Of course FISCHER worked with chaffy fruit coats intact, and it is possible that organic acid had its main function in acting as a culture medium in the carpel wall, so that fungi and bacteria could get a hold on the seed coats. The seed coats seem to form excellent media for the growth of fungi and bacteria. Both layers of cells are very rich in reducing substances. If cultures were started in the carpel wall, they would probably thrive on the seed coats. In contrast to the seed coats, the embryos are very resistant to the attack of fungi and bacteria. As FISCHER points out, one gets good germination in putrefying cultures. This we are quite confident is not due to acid formation, but is rather due to an organism actually decomposing the coats. At 25° C., 0.001 N hydrochloric acid will not cause the rupture of the coats in *Alisma* even after many days' soaking, and this concentration of acid permits but little growth in the naked embryo. It is evident then that the acid could not be strong enough to cause the swelling of the embryo to break the coat and still allow the embryo to grow after it had broken the coat. FISCHER had the preconceived notion that the embryo was dormant, and therefore neglected the possibilities that have proved to be much more to the point. This makes it probable that in putrefying waters in nature the germination is brought about by the direct attack of the seed coats by the organisms, and not through the action of acids formed by the organisms. In the face of the fact that he could soak *Sagittaria* seeds in 1 mol. copper sulphate for 5 days and later secure their germination by treating with acids and placing in water, FISCHER comes to the conclusion that the seed coats are very permeable to solutes in general.

This has been a standing assumption for a long time, but an assumption not backed by evidence. The claim has received lately most telling counter-evidence by the work of BROWN (**5**),. SCHRÖDER (**28**), and SHULL (**29**), from which it appears that semipermeable membranes are of rather common occurrence in seed coats. We have already given evidence for a sodium chloride excluding membrane in the coat of *Alisma*. We find likewise that while the intact seed is not injured by many days' soaking in 1 mol. copper sulphate, the naked embryo will not grow markedly in a 0.001 mol. solution of the same salt.

The conclusions drawn in this paper must be applied only to *Alisma* seeds, but it will be surprising if they do not hold for the very similar seeds of *Sagittaria*, and for the less similar seed of *Eichhornia*, and perhaps for the seeds of many water plants.

The power of acids to hasten the germination of the haw must not be identified with the forcing effects in *Alisma*. In the first case, the effect is on the dormant hypocotyl itself, and in the second, on the coat in the main. In the first case it is hardly necessary to confuse the matter by calling the effect a stimulus, and in the second such an explanation is quite aside from the point.

The peculiar effect of copper sulphate solutions on the germination of acid-treated intact seeds shows the danger of assuming that such effects of salts are on the embryos rather than the coats when intact seeds are used.

We wish to express our gratitude to Dr. SOPHIA ECKERSON for most of the microchemical determinations reported in this paper.

V. Summary

1. Dormancy in the akenes of *Alisma Plantago* is due to the mechanical restraint of the seed coat. This restraint enables the seed to be in water for years without germination.

2. The chaffy carpel wall plays no part in the dormancy. Of the three layers of the seed coat (the outer single layer of reddish-brown cells, the inner single layer of white cells, and the lining acellular pectic hemicellulose material), the outer seems to play no

part in the delay. The effect must be attributed to one or both of the inner layers.

3. As the intact fruit lies in water in the saturated condition, the embryo itself does not half (probably not more than one-fourth or one-fifth) consummate its possible imbibitional and osmotic swelling. The embryo only partly swollen thus lies for years in water, restrained in its swelling by the seed coat, against which it must be exerting a pressure of approximately 100 atmospheres.

4. The air-dry seed (freed from the carpel wall) when placed in water swells rapidly. It increases 40 per cent of its air-dry weight in the course of two hours. From this time on it shows a slow increase to 50 per cent of its air-dry weight, which is maintained constant even after long periods of soaking. A large part of the water absorption is due to the hydrophilous pectic and hemicellulose substances of the seed coat, especially the inner acellular layer.

5. When the coat cap is removed from the large end of the embryo, thus leaving the embryo more free to continue its imbibitional and osmotic swelling, the seed swells even much more rapidly, reaching about 60 per cent of its air-dry weight in two hours and more than 100 per cent after 20 hours. The imbibitional and osmotic swelling gradually passes into growth enlargement.

6. With the coat cap removed from both ends of the embryo and the seed placed in water, the embryo elongates 19 per cent of its air-dry length in 2.5 hours. This would extend the embryo at least 20 per cent the length of the swollen seed beyond the limits of the seed coats. This elongation is all imbibitional and osmotic, involving no growth. Five hours' soaking gives an elongation of 30 per cent of the air-dry length of the embryo. This is only in very small part due to growth. Sixteen hours' soaking gives 36 per cent elongation, which involves considerable growth. Imbibitional and osmotic swelling alone would extend the embryo of *Alisma* far beyond the limits of the swollen seeds.

7. The seed coat is composed almost entirely of pectic substances which are very easily transformed by weak acids and bases. Besides bringing about chemical changes in these substances, acids and bases change their water relations as is true of hydrophilous colloids in general.

8. There is some evidence that acid increases the imbibitional force of the embryo. If such is the case, the increase is very slight. Bases increase greatly the rate of elongation of embryo.

9. It seems that the effect of acids and bases on the germination of *Alisma* seeds is largely to be explained by a weakening of the seed coats, so that the imbibitional and osmotic swelling of the embryo is capable of breaking away the coat cap at the large end of the embryo. It is possible that they are also in part effective by increasing the force of the imbibitional and osmotic swelling.

10. This gives a chemical-physical explanation which displaces the vague implications of the term "stimulus."

11. The seeds of *Alisma*, as of the seeds of water plants in general, are capable of lying in water for years in the imbibed condition without losing their vitality. In contrast to this, seeds of land plants will withstand such storage for a relatively short time.

12. The embryo of *Alisma*, at the expense of its stored foods alone, is capable of more than 120 per cent elongation in total absence of oxygen. For various other phases of its development (greening, branching, development of primary root) it requires some free oxygen. The greening requires at least 5 mm. of air pressure, the branching more than 5 cm. of air pressure, and the general development of primary roots still more.

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